COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Susan Power of Cell & Molecular Technologies, Inc., Phillipsburg, New Jersey, United States of America, declare as follows:

1. At the request of the Patent Attorneys representing Human Genome Sciences ("HGS") in connection with the Ludwig Institute for Cancer Research Opposition to the issuance of HGS Australian Patent Application 696764, in the name of HGS, entitled: "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), I performed certain experiments as described in a Statutory Declaration executed December 13, 2000 ("Power Declaration I"). The Patent Attorneys representing HGS have now requested that I provide additional information regarding those experiments and carry out additional experiments.

2. In particular, I have been asked to clarify the construction of the expression vectors described in Power Declaration I used to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.

3. The Patent Attorneys representing HGS have requested that I perform additional experiments to determine whether the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein

from eukaryotic cells. Further, the Patent Attorneys representing HGS have requested that I construct an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence using only the VEGF-2 coding sequence contained in the ATCC Deposit No. 75698 and the nucleotide sequence of Figure 1 of the HGS application which contains a nucleotide sequence encoding the 350 amino acid form of VEGF-2, and methods and materials known as of March, 1994. I have done this and the experiments I have conducted are described herein.

The Design and Construction of the Expression Vectors Used in the Experiments Described in Power Declaration I

4. The Patent Attorneys representing HGS had previously asked that I perform experiments in order to determine whether the 350 amino acid form of VEGF-2 would be secreted from cells when attached to a heterologous signal sequence. To achieve this aim, I transfected eukaryotic cells with expression vectors encoding either (1) the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or (2) the 419 amino acid form of VEGF-2. The transfected cells were grown and allowed to express the gene products encoded by the vectors. At various time points, both the cell lysates and culture medium were assayed for the presence of VEGF-2 protein. The presence of VEGF-2 protein in either the cell lysates or culture medium was determined by Western Blot analysis of the samples. I have reviewed my notebooks documenting the experiments I performed to achieve the aims of the experiments described in Power Declaration I and provide the following details:

5. For the experiments in Power Declaration I, I was asked to obtain the VEGF-2 DNA directly from the American Tissue Culture Collection (ATCC). I did not obtain any constructs from HGS. The only VEGF-2 clones I obtained were ATCC Deposit No. 97149 and ATCC Deposit No. 75698. The Patent

Attorneys representing HGS provided to me Figure 1 of the HGS patent specification which contains a nucleotide sequence of the 350 amino acid form of VEGF-2. The HGS Patent Attorneys also provided to me the nucleotide sequence of the 419 amino acid form of VEGF-2. It was my understanding that a nucleotide sequence encoding the 350 amino acid form of VEGF-2 was contained in ATCC Deposit No. 75698 and the nucleotide sequence encoding the 419 amino acid form of VEGF-2 was contained in ATCC Deposit No. 97149. It was also my understanding that the amino acid sequence of the 350 amino acid form of VEGF-2 corresponds to residues 70 to 419 of the 419 amino acid form of VEGF-2.

6. As I was under significant time constraints to complete the experiments, I elected to generate the DNA for the expression constructs using only the clone contained in ATCC Deposit No. 97149. Because I was using ATCC Deposit No. 97149 to generate the DNA, I also consulted the nucleotide sequence information relating to the 419 amino acid form of VEGF-2. I considered this to be a reasonable approach since the coding sequences for both the 419 and 350 amino acid forms of VEGF-2 are contained in ATCC Deposit No. 97149. Thus, I isolated the nucleotide sequences encoding the 419 amino acid form of VEGF-2 as well as the 350 amino acid form of VEGF-2 using ATCC Deposit No. 97149 as the sole source of VEGF-2 coding sequences.

7. My understanding of the goals of the experiments described in Power Declaration I was to demonstrate that the 350 amino acid form of VEGF-2 could be successfully expressed and secreted when expressed as taught by the HGS patent specification, *i.e.*, using a heterologous signal sequence. I did not inform the patent attorneys representing HGS at the time of carrying out these experiments nor at the time of signing Power Declaration I that I had isolated the 350 amino acid form of VEGF-2 from the ATCC Deposit No. 97149 clone. It was only when they asked on or about September 24, 2001 for

further clarification of the experiments that I conducted that I informed them of these details.

8. I have now been asked to redesign my experimental protocol to specifically use the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to generate the expression construct containing the 350 amino acid form of VEGF-2. I have been asked that I perform the experiments using the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.

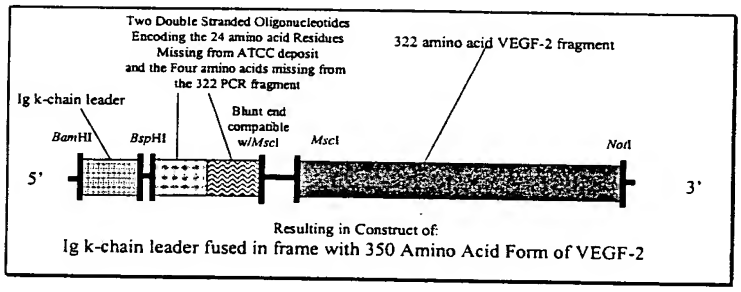
9. I have provided the Patent Attorneys for HGS with the details of a sequence analysis of the VEGF-2 coding sequence contained in ATCC Deposit No. 75698. The VEGF-2 clone contained in the ATCC Deposit No. 75698 lacks 24 amino acids at the N-terminal end of the 350 amino acid form of VEGF-2, and corresponds to residues 94 to 419 of the 419 amino acid form of VEGF-2, *i.e.*, a 326 amino acid form of VEGF-2. I have also been asked to perform experiments to determine if the 326 amino acid form of VEGF-2 as encoded by a nucleotide sequence contained in ATCC Deposit No. 75698 fused to a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.

10. Even though ATCC Deposit No. 75698 lacks the complete coding sequence for the 350 amino acid form of VEGF-2, a molecular biologist as of March 1994 would be able to recreate the 350 amino acid form of VEGF-2 given the description of the complete sequence in the HGS patent specification (as described below) and that is the course I could have taken at that time and I would have expected other molecular biologists to have been able to do the same. I generated an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, using only the

ATCC Deposit No. 75698 and the sequence of Figure 1 in the HGS patent specification, and techniques and materials routinely known and used in the art as of March 1994.

The Expression Vector Containing the 350 Amino Acid Form of VEGF-2 Is Generated Using Only ATCC Deposit No. 75698 and Figure 1 of the HGS Patent Specification

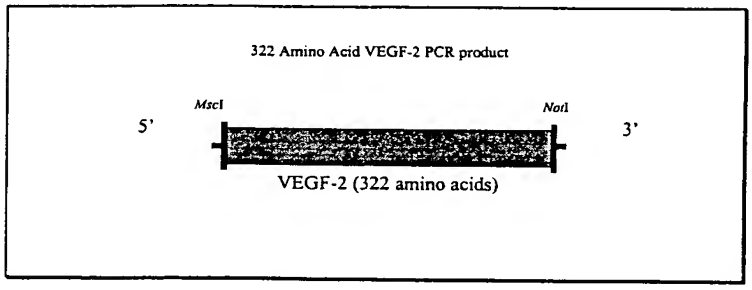
11. The general design of the expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence is as follows:



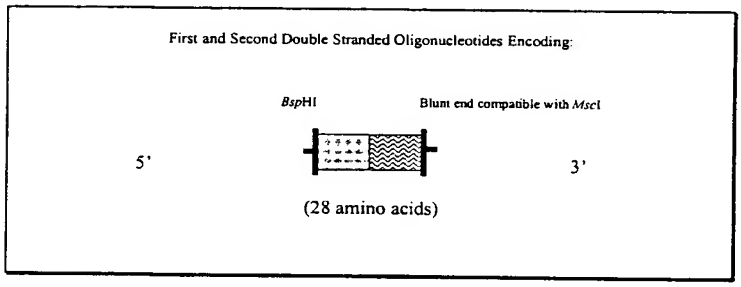
12. Since all that I had at my disposal were ATCC Deposit No. 75698 and Figure 1 of the HGS patent specification, I did the following:

12.1 First, I chose to directly isolate a nucleotide sequence encoding the C-terminal 322 residues of the 326 amino acid form of VEGF-2. The 322 residues corresponding to residues 98 to 419 of the 419 amino acid form of VEGF-2 were amplified by PCR from ATCC Deposit No. 75698. I chose to isolate a VEGF-2 fragment of 322 amino acids to facilitate the cloning of the VEGF-2 coding sequence in frame into the expression constructs. To do so, I designed primers based on the sequence provided in Figure 1 of the HGS patent specification, the sequence of ATCC Deposit No. 75698, and the sequence of restriction enzyme recognition sites, *e.g.*, *Msc*I and *Not*I. The resulting 322 amino

acid fragment of VEGF-2 amplified from ATCC Deposit No. 75698 was digested with *Msc* I and *Not* I.

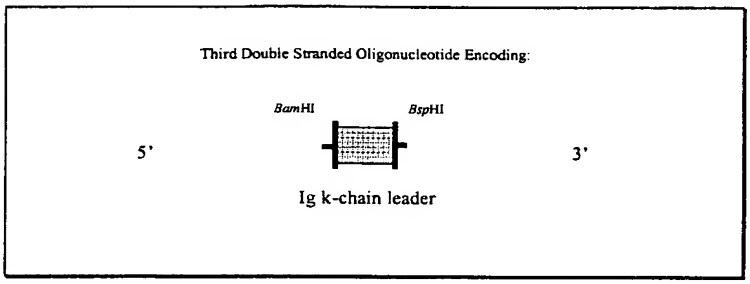


12.2 Using a nucleotide sequence encoding the 350 amino acid form of VEGF-2 contained in Figure 1 of the HGS specification, I designed two double stranded oligonucleotides to encode (once ligated together) a 28 amino acid VEGF-2 fragment. This fragment encompasses the 24 amino acids missing from ATCC Deposit No. 75698 and the additional 4 amino acids missing from the 322 amino acid fragment of the 326 form of VEGF-2. Specifically, once ligated together, the oligonucleotides were designed to result in the generation of a 28 amino acid fragment engineered to have a 5' end with a *Bsp*H I restriction site overhang and a 3' blunt end compatible with a *Msc* I restriction site as shown below. Methods and materials for generating such double stranded oligonucleotides were routine and known by March, 1994.



12.3 A third double stranded oligonucleotide encoding the secretion signal sequence of the Ig k-chain leader signal sequence that was also used in

the experiments described in Power Declaration I was engineered to contain a *Bam* HI restriction enzyme overhang at the 5' end and a *Bsp* HI restriction enzyme overhang at the 3' end as shown below. Ig k-chain leader signal sequence was a recognized signal sequence available as of March, 1994.



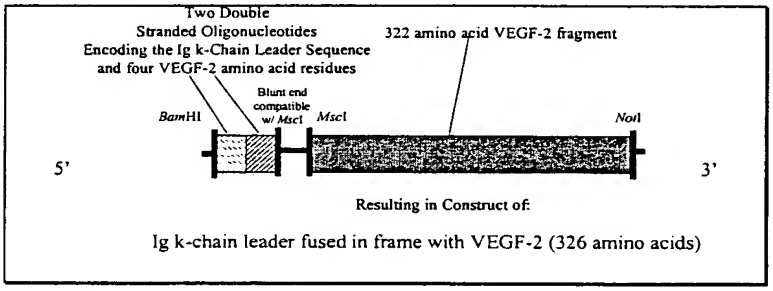
12.4 The 322 amino acid VEGF-2 fragment and the three double stranded oligonucleotides described above were ligated and subcloned at once into the *Not* I/*Bam* HI sites of the expression vector pCMV-I which was described in Power Declaration I. The resulting expression vector contains the construct as described in ¶ 11 above. The VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March, 1994.

12.5 The resulting 350 amino acid form of VEGF-2 construct was sequenced and confirmed to be correct and is detailed in Appendix I.

13. The design of the expression vector containing the VEGF-2 coding sequence found in ATCC Deposit No. 75698 used in the study results in a construct with the 326 amino acid form of VEGF-2 linked to a heterologous sequence and is as follows:

14. To generate the construct, the 322 amino acid VEGF-2 fragment flanked with a *Msc* I site at the 5' end and the *Not* I site at the 3' end was generated as

described above (*see* ¶12.1). I designed two double stranded oliogonucleotides that once ligated together encoded the Ig k-chain leader signal sequence and the four amino acid residues corresponding to residues 94 to 97 of the 419 amino acid form of VEGF-2, *i.e.,* the first four residues of the 326 amino acid form of VEGF-2 of ATCC Deposit No. 75698 engineered to contain a 3' blunt end compatible with a *Msc*I restriction site and a 5' *Bam* HI site. The 322 amino acid VEGF-2 fragment was simultaneously fused in frame with the two double stranded oliogonucleotides, as shown below, and subcloned into the expression vector pCMV-I *Bam* HI/ *Not* I sites. Again, the VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March 1994.



15. The sequence of the resulting 326 amino acid form of VEGF-2 construct was confirmed to be correct and is detailed in Appendix II, attached hereto.

16. For purposes of the following experiments, I used the expression vector encoding the 419 amino acid form of VEGF-2 described in Power Declaration I (*see* Power Declaration I ¶¶ 3 to 6).

17. As set out in Power Declaration I, the sequence of the construct was confirmed to be correct and is detailed in Appendix III, attached hereto.

Using Only the VEGF-2 Clone Contained in ATCC Deposit No. 75698 Fused in Frame with a Heterologous Signal Sequence, Expression and Secretion of VEGF-2 Is Achieved

18. The Patent Attorneys for HGS requested that I perform the following experiments in order to determine whether using only the 350 amino acid form or the 326 amino acid form of VEGF-2 contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of VEGF-2 from eukaryotic cells.

19. The overall experimental design is as follows: eukaryotic cells were transfected with expression vectors encoding the 419 amino acid form of VEGF-2, the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or the 326 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence. The transfected cells were cultured for 24 or 48 hours to allow for expression of the gene products encoded by each vector. In order to determine whether the VEGF-2 gene product was being expressed and secreted, the cell lysates and culture medium were collected to assay for the presence of VEGF-2. The presence of VEGF-2 in either the cell lysates or culture medium was determined by Western Blot analysis of the samples using a rabbit polyclonal antibody to VEGF-2 that recognizes all forms and fragments of VEGF-2. The same antibody was used in Power Declaration I to assay the presence of VEGF-2 proteins.

20. The three VEGF-2 constructs encoding the 419, 350 and 326 amino acid forms of VEGF-2 each were transiently transfected in duplicate, using the lipofectin method into the Human Embryonic Kidney cell line, HEK-293 tsA-0. The method of transfection and the cell line were both routinely used as of March, 1994. As a control for transfection efficiency, each construct was co-transfected with the vector pCMV-β-gal. The efficiency of transfection was determined by β-gal staining 48 hours after transfection. As a negative control,

the vector pCMV-I without the addition of any VEGF-2 coding sequences was transfected in parallel.

21. The transfection design is as follows:

6 dishes transfected with: pCMV-I-VEGF-419;

6 dishes transfected with: pCMV-I-signal sequence-VEGF-350;

6 dishes transfected with: pCMV-I-signal sequence-VEGF-326;

6 dishes transfected: pCMV-I;

1 dish transfected with: pCMV-I-VEGF-419 + pCMV-β-gal;

1 dish transfected with: pCMV-I-signal sequence-VEGF-350 + pCMV-β-gal; and

1 dish transfected with: pCMV-I-signal sequence-VEGF-326 + pCMV-β-gal.

22. After transfection, DMEM medium containing 3% serum was added to the cells. Aliquots of cell extracts and conditioned medium were prepared from each transfection at: T_0 hours, T_{24} hours and T_{48} hours, in duplicate.

23. At the time of harvesting the cells and medium were treated as follows:

Medium: Harvested medium was concentrated 3 fold using Centricon 10 concentrator devices. One volume of 2 x PAGE loading dye was added to each sample.

Cell Extracts: The cells were harvested by trypsinization and collected by centrifugation. The cell pellet was resuspended and lysed, and one volume of 2 x PAGE loading dye was added to each sample.

24. To determine the transfection efficiency, dishes transfected with the pCMV-β-gal construct were fixed and stained for β-gal activity 48 hours after transfection. All dishes were found to have the same percentage of transfected cells (70%).

25. Each protein sample was subjected to Western analysis as outlined below. Prior to loading on to a 12% (w/v) Tris-Glycine SDS-Polyacrylamide gel, the samples were boiled for 5 minutes and cooled on ice. The two end lanes of each gel contained the appropriately sized molecular weight markers to estimate the migration rate of proteins predicted to run in the 16 to 85 kDa size range. The samples were electrophoresed according to standard conditions.

26. Following electrophoresis, the samples were transferred to a PVDF membrane. Each membrane was blocked by a one hour incubation in phosphate buffered saline (PBS) containing 3% Bovine Serum Albumin (BSA). The blot was then incubated at 4°C in PBS containing 0.1% BSA and 500ng/ml of purified rabbit anti-VEGF-2 antibody, a polyclonal antibody which recognizes all immunogenic fragments of VEGF-2. After three 5 minute washes in PBS containing 0.1% Tween, the blot was incubated for 1 hour in PBS containing 0.1% BSA and a 1:3000 dilution of Goat Anti-Rabbit IgG Horse Radish Peroxidase (HRP) conjugated antibody. The blot was washed six times for 5 minutes in PBS containing 0.1% BSA. The blot was developed with 2ml/blot of ECL detection reagent (obtained from Amersham) for one minute and then exposed directly to Polaroid films for approximately 2-3 seconds.

27. The result of the experiment is shown in Figure 1, attached hereto. The samples included in the figure are as follows:

Immunoblot analysis of VEGF constructs transiently expressed in HEK293T cells

Lane	**Pellet/ Supernatant**	**Construct (419, 350, 326, or neg. control)**	**T (h) post-transfection**
Gel 1			
1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24

6	S	326-signal	24
7	P	419	48
8	S	419	48
Gel 2			
1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24
6	S	326-signal	24
7	P	419	48
8	S	419	48
Gel 3			
1	P	Negative control	24
2	S	Negative control	24
3	P	419	24
4	S	419	24
5	P	419	24
6	S	419	24
7	P	350-signal	48
8	S	350-signal	48
9	P	419	48
10	S	419	48
Gel 4			
1	P	Negative control	48
2	S	Negative control	48
3	P	350-signal	48
4	S	350-signal	48
5	P	326-signal	48
6	S	326-signal	48
7	P	326-signal	48
8	S	326-signal	48

9	P	419	48
10	S	419	48

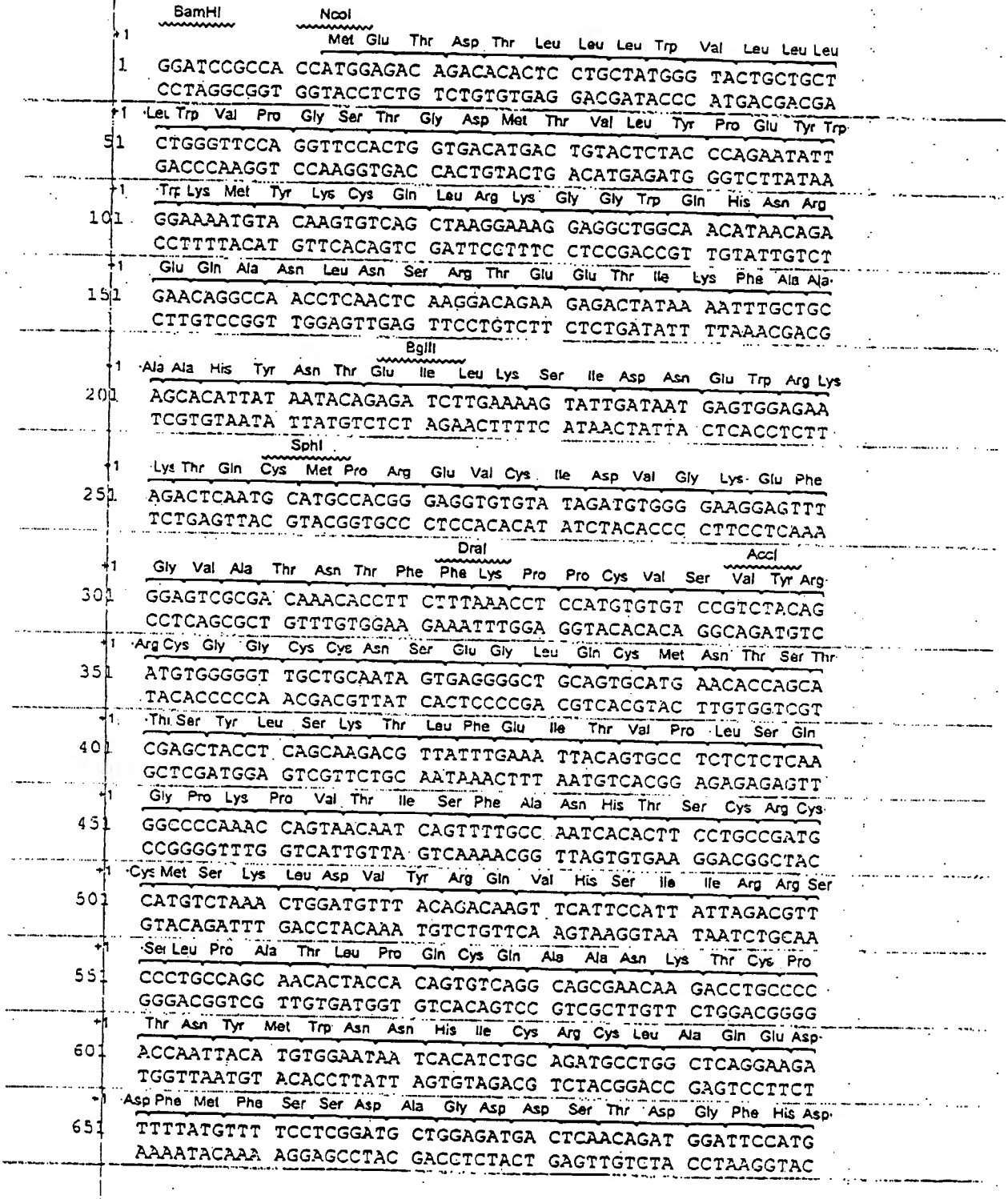
31. The Western Blot analysis indicates a broad band resolving at approximately 30kDa was present in the medium collected from the transfection of the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF-2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct (see Figure 1, attached hereto as Appendix IV). The secreted protein was visible at 24 hours and 48 hours after transfection. The secreted product from cells containing the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct are all the same approximate size.

AND I declare that all the statements made in this Declaration are of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Susan Power, Susan Power at Phillipsburg, New Jersey, on this 22nd day of March 2002;
before me Gean Rotmistrenko
Notary Public

GEAN ROTMISTRENKO
Notary Public, State of New York
No. 41-4778718
Qualified in Queens County
Certificate Filed in New York County
Commission Expires October 31, 2025

VEGF-350+Signal



POWER DECLARATION II

APPENDIX I

VEGF-350+Signal

```
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      TGTAGACACC TGGTTTGTTC CTCGACCTAC TTCTCTGGAC AGTCACACAG

                    Bsr8I
                +1  Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp
 751  TGCAGAGCGG GGCTTCGGCC TGCCAGCTGT GGACCCCACA AAGAACTAGA
      ACGTCTCGCC CCGAAGCCGG ACGGTCGACA CCTGGGGTGT TTCTTGATCT

                +1  Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys
 801  CAGAAACTCA TGCCAGTGTG TCTGTAAAAA CAAACTCTTC CCCAGCCAAT
      GTCTTTGAGT ACGGTCACAC AGACATTTTT GTTTGAGAAG GGGTCGGTTA

                +1  Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys
 851  GTGGGGCCAA CCGAGAATTT GATGAAAACA CATGCCAGTG TGTATGTAAA
      CACCCCGGTT GGCTCTTAAA CTACTTTTGT GTACGGTCAC ACATACATTT

                +1  Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu
 901  AGAACCTGCC CCAGAAATCA ACCCCTAAAT CCTGGAAAAT GTGCCTGTGA
      TCTTGGACGG GGTCTTTAGT TGGGGATTTA GGACCTTTTA CACGGACACT

                +1  Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His
 951  ATGTACAGAA AGTCCACAGA AATGCTTGTT AAAAGGAAAG AAGTTCCACC
      TACATGTCTT TCAGGTGTCT TTACGAACAA TTTTCCTTTC TTCAAGGTGG

                +1  His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala
1001  ACCAAACATG CAGCTGTTAC AGACGGCCAT GTACGAACCG CCAGAAGGCT
      TGGTTTGTAC GTCGACAATG TCTGCCGGTA CATGCTTGGC GGTCTTCCGA

                +1  Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser
1051  TGTGAGCCAG GATTTTCATA TAGTGAAGAA GTGTGTCGTT GCGTCCCTTC
      ACACTCGGTC CTAAAAGTAT ATCACTTCTT CACACAGCAA CGCAGGGAAG

                                                    NotI
                                                    EagI
                +1  Ser Tyr Trp Lys Arg Pro Gln Met Ser ---
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      TATAACCTTT TCTGGTGTTT ACTCGATTCG CCGGCGC
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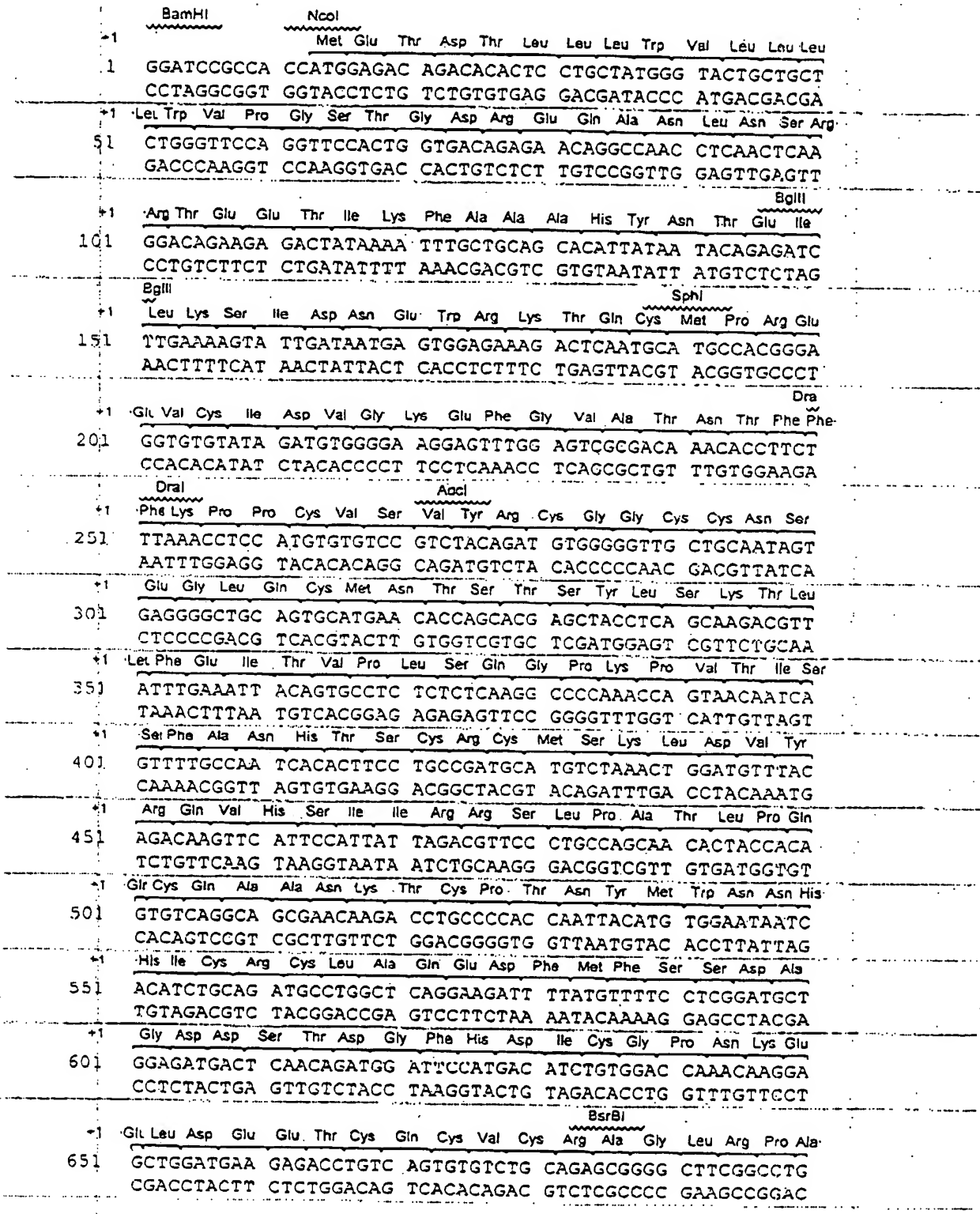
VEGF 326+Signal



POWER DECLARATION II

APPENDIX II

VEGF 326+Signal

```
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  701     CCAGCTGTGG ACCCCACAAA GAACTAGACA GAAACTCATG CCAGTGTGTC
          GGTCGACACC TGGGGTGTTT CTTGATCTGT CTTTGAGTAC GGTCACACAG
      +1  Cys Lys Asn  Lys  Leu Phe  Pro  Ser Gln  Cys  Gly Ala Asn  Arg  Glu Phe Asp
  751     TGTAAAAACA AACTCTTCCC CAGCCAATGT GGGGCCAACC GAGAATTTGA
          ACATTTTTGT TTGAGAAGGG GTCGGTTACA CCCCGGTTGG CTCTTAAACT
      +1 Asp Glu Asn  Thr  Cys Gln Cys  Val  Cys Lys  Arg  Thr Cys  Pro  Arg Asn Gln Pro
  801     TGAAAACACA TGCCAGTGTG TATGTAAAAG AACCTGCCCC AGAAATCAAC
          ACTTTTGTGT ACGGTCACAC ATACATTTTC TTGGACGGGG TCTTTAGTTG
                                                    BsrGI
      +1  Pro Leu Asn  Pro  Gly Lys  Cys  Ala Cys Glu  Cys  Thr Glu  Ser  Pro Gln Lys
  851     CCCTAAATCC TGGAAAATGT GCCTGTGAAT GTACAGAAAG TCCACAGAAA
          GGGATTTAGG ACCTTTTACA CGGACACTTA CATGTCTTTC AGGTGTCTTT
      +1  Cys Leu Leu  Lys  Gly Lys  Lys  Phe His  His  Gln Thr Cys  Ser  Cys Tyr Arg
  901     TGCTTGTTAA AAGGAAAGAA GTTCCACCAC CAAACATGCA GCTGTTACAG
          ACGAACAATT TTCCTTTCTT CAAGGTGGTG GTTTGTACGT CGACAATGTC
      +1 Arg Arg  Pro  Cys  Thr Asn Arg  Gln  Lys  Ala  Cys  Glu  Pro  Gly  Phe Ser  Tyr Ser
  951     ACGGCCATGT ACGAACCGCC AGAAGGCTTG TGAGCCAGGA TTTTCATATA
          TGCCGGTACA TGCTTGGCGG TCTTCCGAAC ACTCGGTCCT AAAAGTATAT
      +1  Ser Glu Glu  Val  Cys Arg  Cys  Val Pro  Ser  Tyr  Trp Lys  Arg  Pro Gln Met
 1001     GTGAAGAAGT GTGTCGTTGT GTCCCTTCAT ATTGGAAAAG ACCACAAATG
          CACTTCTTCA CACAGCAACA CAGGGAAGTA TAACCTTTTC TGGTGTTTAC
                  NotI
                  EagI
      +1  Ser ***
 1051     AGCTAAGCGG CCGCG
          TCGATTCGCC GGCGC
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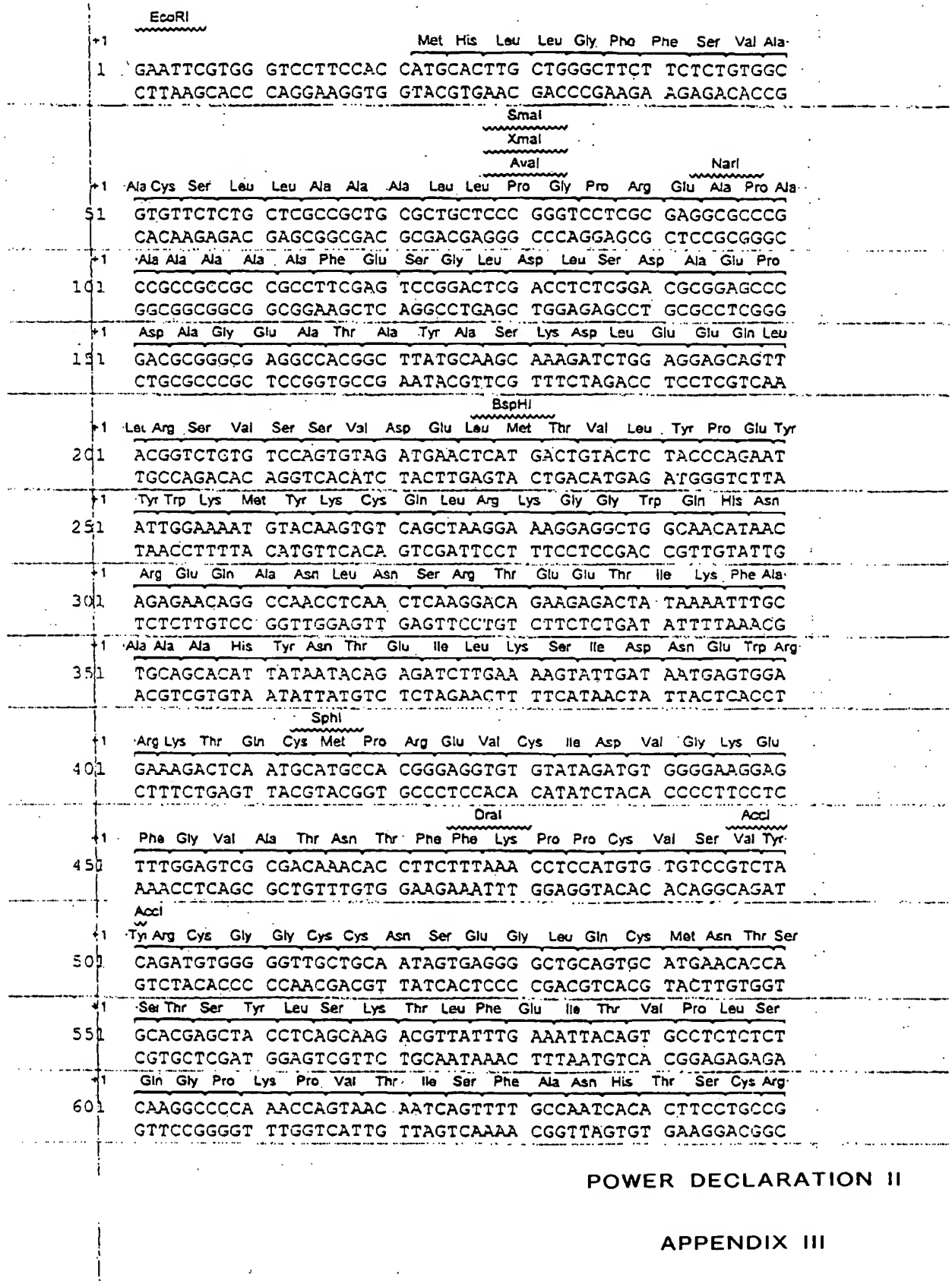
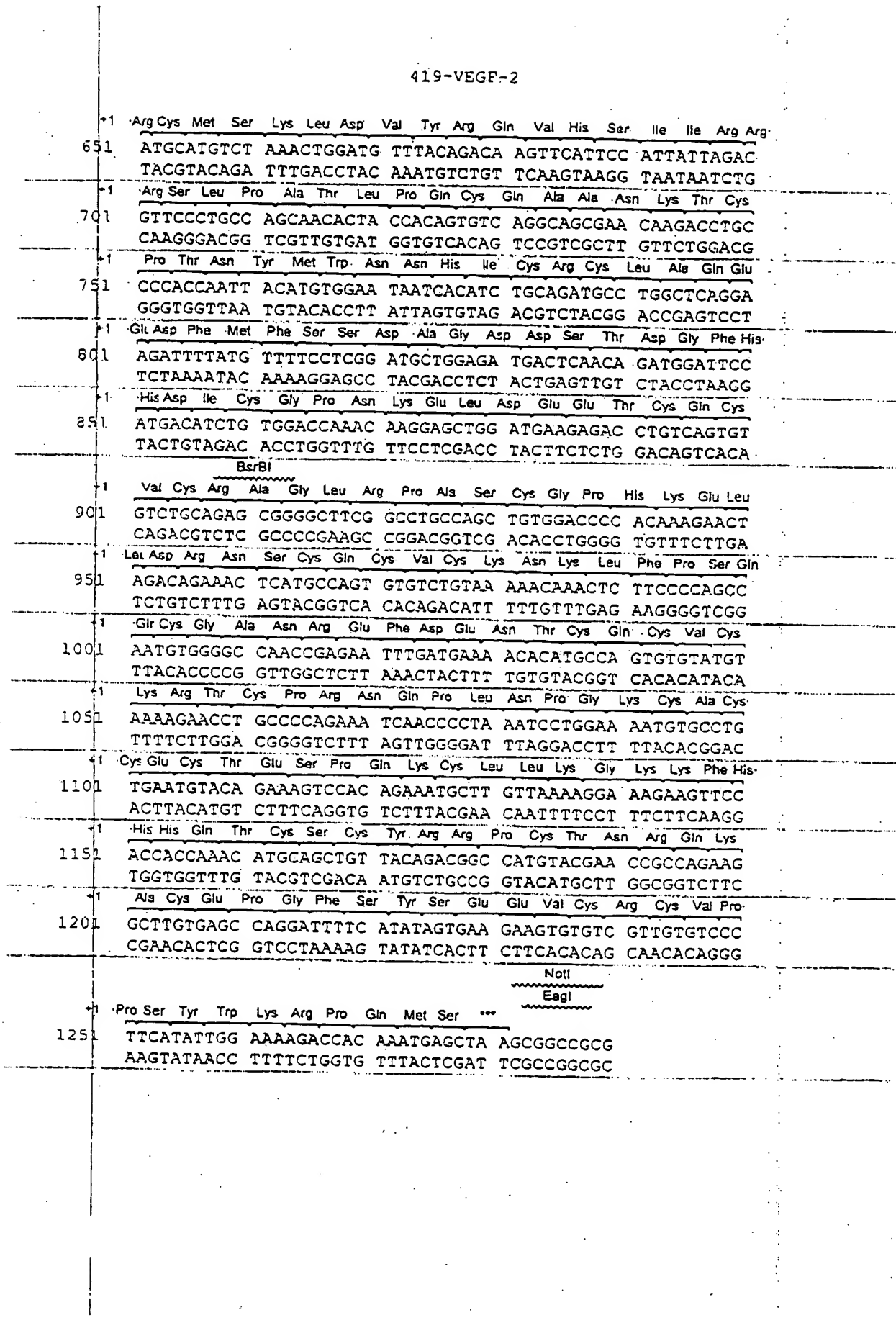


POWER DECLARATION II

APPENDIX III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+1 Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys
701 GTTCCCTGCC AGCAACACTA CCACAGTGTC AGGCAGCGAA CAAGACCTGC
CAAGGGACGG TCGTTGTGAT GGTGTCACAG TCCGTCGCTT GTTCTGGACG
+1 Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu
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+1 Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
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+1 His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
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BsrBI
+1 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu
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+1 His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys
1151 ACCACCAAAC ATGCAGCTGT TACAGACGGC CATGTACGAA CCGCCAGAAG
TGGTGGTTTG TACGTCGACA ATGTCTGCCG GTACATGCTT GGCGGTCTTC
+1 Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro
1201 GCTTGTGAGC CAGGATTTTC ATATAGTGAA GAAGTGTGTC GTTGTGTCCC
CGAACACTCG GTCCTAAAAG TATATCACTT CTTCACACAG CAACACAGGG
NotI
EagI
+1 Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser •••
1251 TTCATATTGG AAAAGACCAC AAATGAGCTA AGCGGCCGCG
AAGTATAACC TTTTCTGGTG TTTACTCGAT TCGCCGGCGC